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13. ABSTRACT (Maximum 200 Words)

Sonic hedgehog(shh)overexpression in LNCaP cells accelerates tumor growth. To characterize the contributions of each Gli gene to Shh-induced tumor growth, Gli1, Gli2 and Gli3 knockout UGS-M cells have been isolated and UGSM-2 cells were transfected with the Gli expression vectors. Gli1 and Gli2 gene were overexpressed in the cell line, and we have confirmed activation of the downstream target genes Patch and Hip in the Gli2 overexpressed cells. Experiments using those cell lines in the xenograft model are currently being done.

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Prostate cancer, tumor, sonic hedgehog signal, Gli

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Introduction:

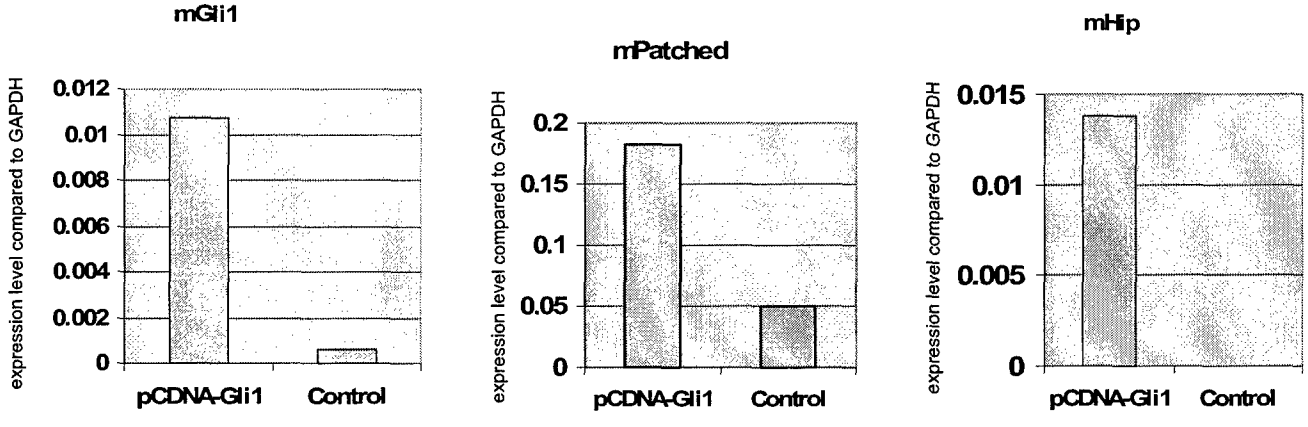
Activation stroma-mediated paracrine signals by epithelial sonic hedgehog expression to promote tumor growth has been first demonstrated in our lab. To test the hypothesis that activation of stromal Gli gene expression is necessary and sufficient to promote tumor growth and elucidate the roles of the three different Gli genes in the stromal response to Shh signaling, we planned to examine the effects of gain and loss function of each Gli gene using a well characterized Xenograft model.

Results

As proposed in Specific Aim 1, We have transfected the UGSM-2 cells with the Gli gene expression vectors and verified Gli1 and Gli2 gene overexpression. Characterization of those cells showed that Patched and Hip genes were activated, these means Gli1 and Gli2 protein were functional in those cells. For testing the more improtance of paracrine signal compared to autocrine signal of shh pathway in tumor growth, we are currently generating Gli1 or Gli2 overexpress LNCap cells.

I inserted the coding sequence of human Gli1 gene into the Vector pCDNA3.1/Hygro(+), transfected the UGSM-2 cells with pCDNA-Gli1 using Lipofectomine 2000, cells transfected with pCDNA3.1/Hygro(+) as control. 200ug/ml hygromycin were added into the medium as the selection marker. Quantitative real time PCR results were shown in Figure1A, in theUGSM-2 cells overexpressing Gli1, the expression level of mGli1 gene is about 18 fold higher, mPatched gene is 3.6 fold higher and Hip gene is 130000 fold (the basic level of mHip gene is extremely low compared to GAPDH).

Under hygromycin selection, the UGSM-2 cells became smaller than the normal cells , so the coding sequence of Human Gli2ß gene was inserted to pIRES-EGFP. Two days after transfection, cells were trypsinized and isolated by sterile cell sorting on a FACs Vantage SE cell sorter equipped with FACS Diva Option software based on the green fluorescence of the transfected cells. Figure 1B shows that mGli2 gene is overexpressed (19 fold higher than the control) in the cells and the expression of mPatched gene and mHip gene were also induced.



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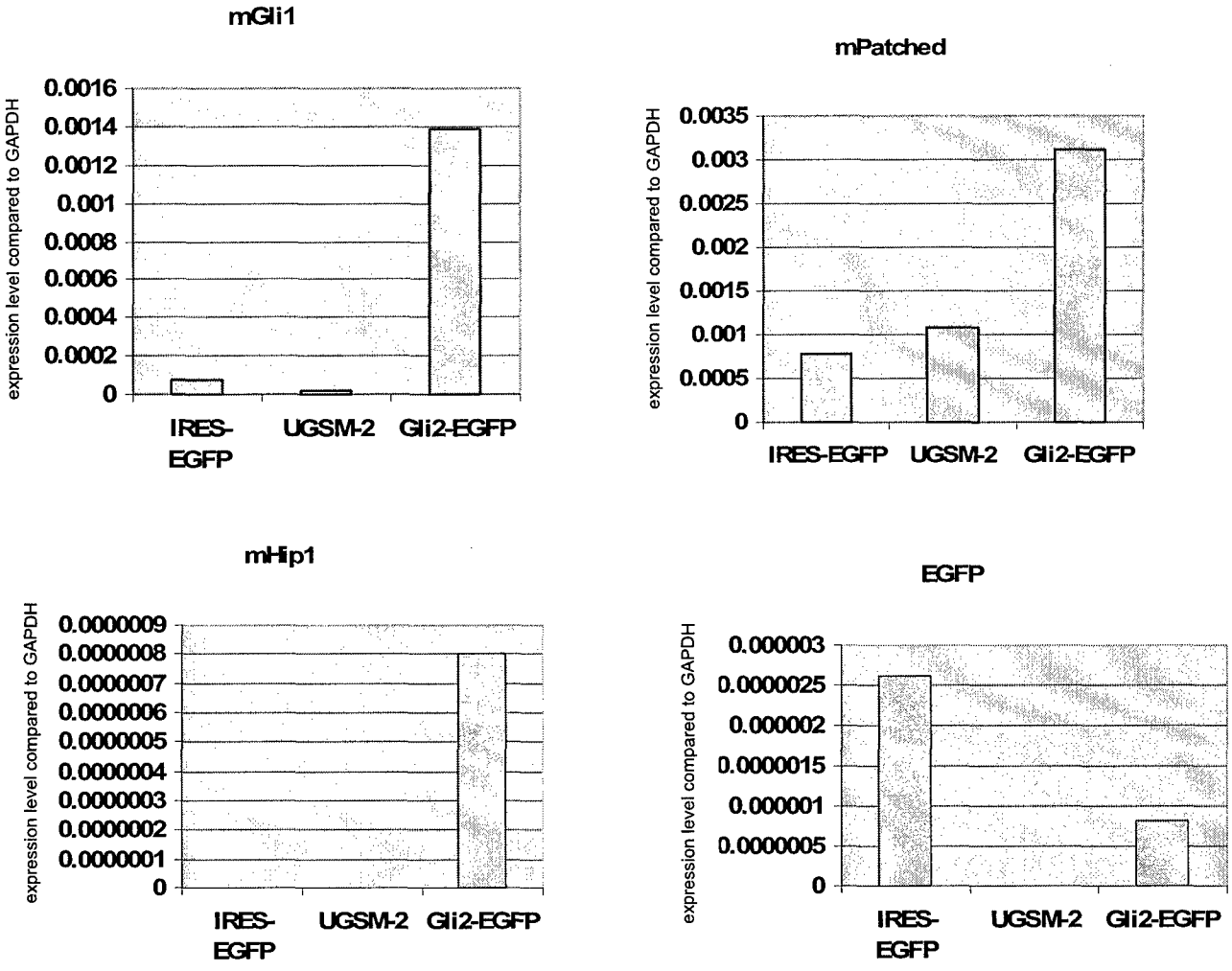

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Figure 1 Real time PCR shows overexpression of human Gli1 and Gli2 gene in mouse UGSM-2 cells. Mouse Gli1 was overexpressed in UGSM-2 cells transfected with pCDNA-Gli1. and downstream target genes Patched and Hip were also activated (Figure1A). Mouse Gli1, Patched and Hip were overexpressed in the UGSM cells overexpressing Gli2, GFP was a selection marker in these cells (Figure 1B), cells transfected with pIRES-EGFP was a control.

As proposed in Specific Aim2, We have islolated Gli1, Gli2 and Gli3 knockout UGSM cells respectively. We are using those knockout cell lines with LNCaP cells and LNCaP cells which overexpress shh for our Xenograft model research.

Heterozygous $Gli1^{(+/-)}$, $Gli2^{(+/-)}$ and $Gli3^{(+/-)}$ mice (INK4A homozygous background) were bred to generate $Gli1^{(-/-)}$, $Gli2^{(-/-)}$ and $Gli3^{(-/-)}$knockout embryos. Male mouse embryos were harvested at 16 days post-conception and the prostate mesenchymal cell layer were isolated by trysin and collagenase digestion to recover single cells. These cells were grown in culture where they spontaneously immortalize due to the INK4A (P16, P19 knock out) background. These cell lines have been characterized by quantitative real time PCR.

$Gli1^{(-/-)}$, $Gli2^{(-/-)}$ and $Gli3^{(-/-)}$ knockout cells were treated with shh and cyclopamine (shh pathway inhibitor). Quantitative real time PCR results confirmed that these knockout cells still respond to shh and cyclopamine, exogenous shh can activate gene expression of gli1 and patched, and cyclopamine can block the activation (Figure 2, 3 and 4).

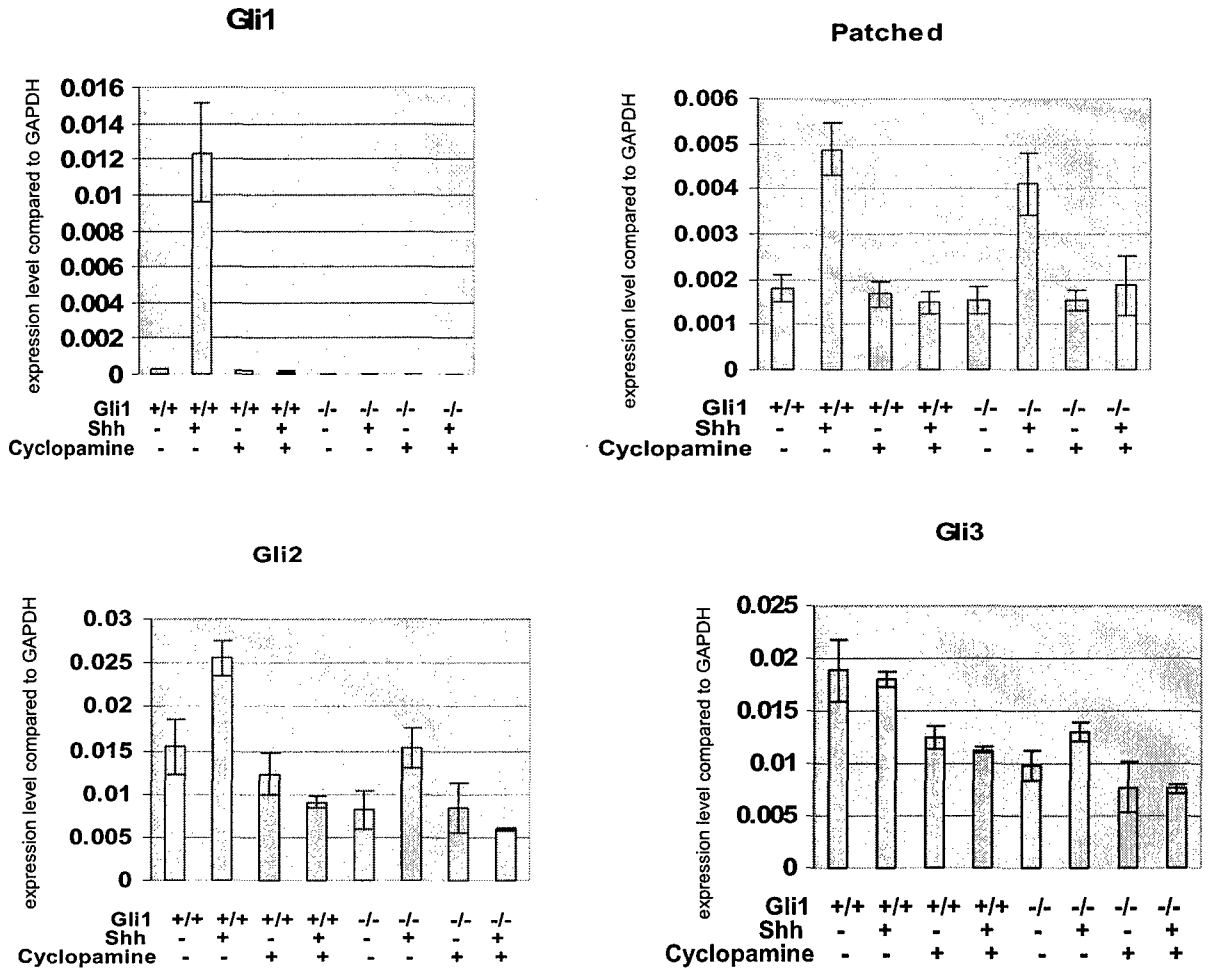
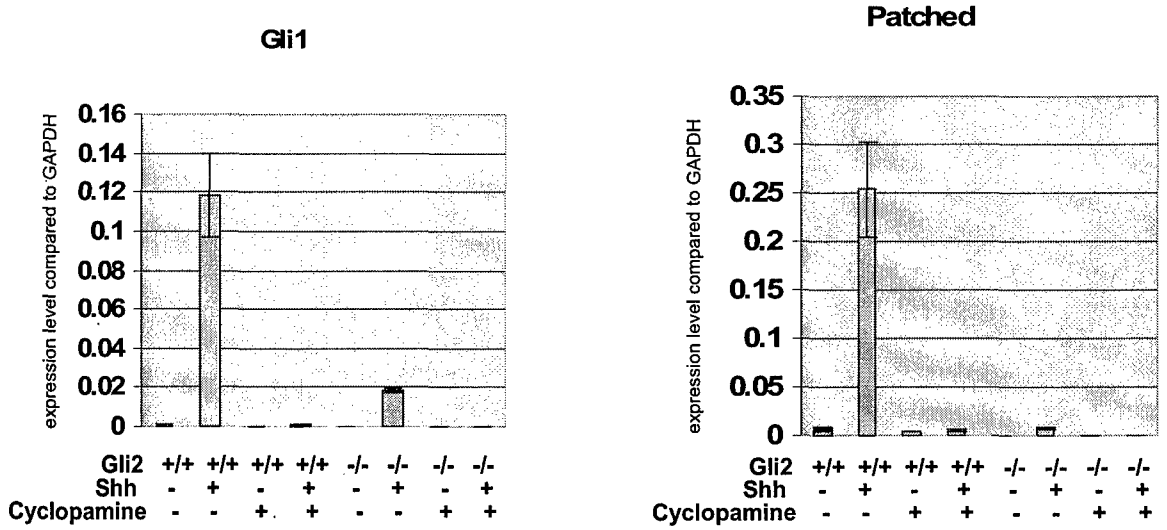


Figure 2 The response of Gli1$^{(-/-)}$ knockout cells to shh and cyclopamine. Cells were treated with 1nM shh and 5μM cyclopamine. The gene expression level was all compared to GAPDH. No gli1 expression was detected in the knockout cells. The expression of gli2 and gli3 are similar in the knockout cells compared to wild type cells.



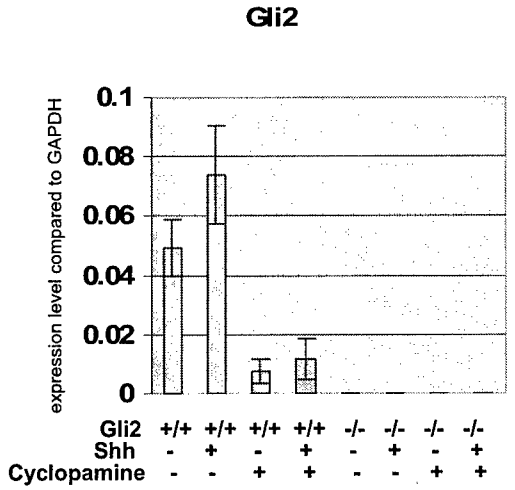
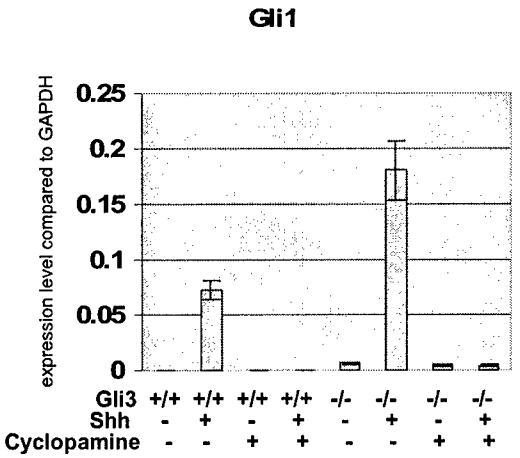
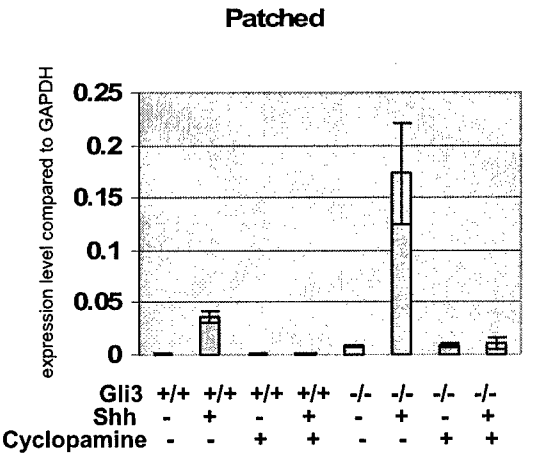
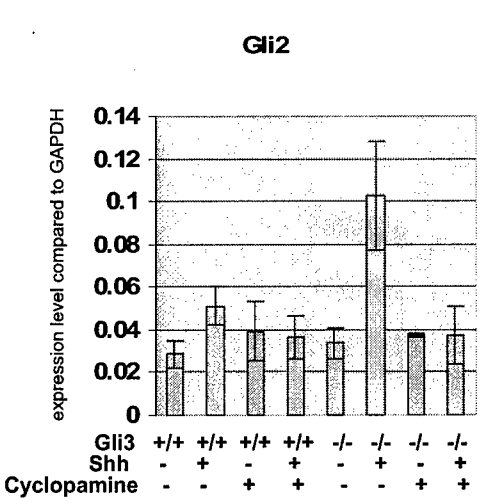


Figure 3 Gli2$^{(-/-)}$ knockout cells respond to shh and cyclopamine. Cells were treated as same as Gli1$^{(-/-)}$ knockout cells. Gli2 gene is not expressing in the knockout cells. The expression of gli1 and Patched are similar in the knockout cells compared to wild type cells.







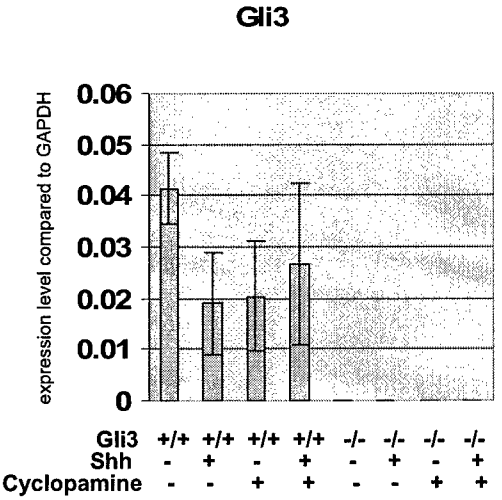


Figure 4 The response of Gli3$^{(-/-)}$ knockout cells to shh and cyclopamine. Cells were treated as before. Gli3 expression was not detected in the knockout cells. The expression of gli1 and patched are about 2-3 fold higher

in the knockout cells , it means that gli3 works as a negative regulator in the shh pathway of mouse UGSM cells.

Key Research Accomplishments:

- We have developed genetically modified stromal cell lines to assay the specific roles of Gli1, Gli2, Gli3 in tumor growth.

- We have generated and characterized Gli1 and Gli2 overexpression UGSM-2 cells and shown that Patched and Hip genes were activated by Gli1 and Gli2.

Training experiences

- I learned mammalian cell culture, transfection, flow cytometry by overexpression of Gli genes in the UGSM cells.
- I also learned to ring clone and to isolate clones by islation of Gli, Gli2, Gli3 knockout UGSM cells. Isolation of clonal cell populations is important in recovering cells which express the gene of interest at variable levels.
- In addition, I have used Brdu labeling of cells in culture to monitor cell proliferation following transfection of Gli genes.

Reportable Outcomes:

Gli1, Gli2, Gli3 knock out UGSM cell lines
UGSM cell lines that overexpress Gli1 or Gli2
A manuscript has been submitted. R.J.Lipinski, J.Zhang. J.Gipp, J.D.Doles and W.Bushman: Individual and Cooperative Activities of the Gli Transcription Factors in Hedgehog Signaling.

Conclusions:

We are making progress on the approved aims of the grant and obtaining data which, to this point, validates the research hypothesis.

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Appendics
None